

Figure 2. DNA and amino acid sequences of the mouse 21.6 heavy chain variable region.

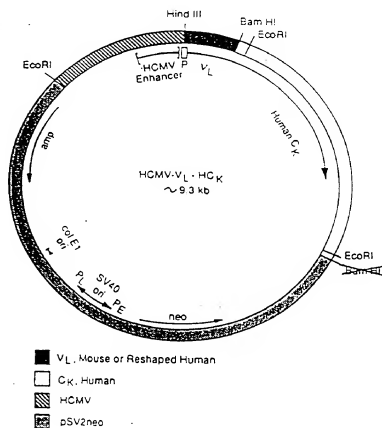
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1  atgaaatgcagctgggtcatgttcttctctgatggcagtggttacaggggtcaattcagag  60
   tactttacgtcgaccagtacaagaaggactaccgtcaccaatgtccccagttaagttc
ACTAGTCGACATGAAATGCAGCTGGGTCACTTCTTC-3'
                                G
MHV1 Primer
[M K C S W V M F F L M A V V T G V N S][E -
                                Leader
61  gttcagctcgacagctctggggcagagcttgtgaagccaggggcctcagtcagggtgtcc  120
   caagtcgacgtcgctcagaccccgctctcgaacacttcgggtccccggagtcagttcaacagg
   V Q L Q Q S G A E L V K P G A S V K L S -
                                FR1
121  tgcacagctcttctggcttcaacattaaagacacctatatacacctgtgtgaagcagaggcct  180
   acgtgtcgaagaccgaagtgttaatttctgtggatatatgtgacacacttcgtctccgga
   C T A S G F N I K][D T Y I H][C V K Q R P -
                                CDR1
181  gaacagggcctggagtggttggaaggattgatccctgcgaatggttataactaaatgatgac  240
   cttgtcccggaacctcacctaaccttccctaactaggacgcttaccaatatgatattatactg
   E Q G L E W I G][R I D P A N G Y T K Y D -
                                FR2                                CDR2
241  ccgaagttccaggggcaaggccactataacagctgacacatcctccaacacagcctacctg  300
   ggcttcaagggtcccggttcgggtgatattgtcgactgtgtaggaggtgtgtcggtatggac
   P K F Q G][K A T I T A D T S S N T A Y L -
301  cagctcagcagcctgacatctgaggacactgccgtctatttctgtgctagagagggatatt  360
   gtcgagtcgtcggaactgttagactcctgtgacggcagataaagacacgactctctccctata
   Q L S S L T S E D T A V Y F C A R][E G Y -
                                FR3
361  tatggttaactacggggctctatgctatggactactgggggtcaaggaaacctcagtcaccgtc  420
   ataccattgatccccagatacgcatacctgatgacccaggttccttggagtcagtgccag
   Y G N Y G V Y A M D Y][W G Q G T S V T V -
                                CDR3                                FR4
                                Mouse Gamma-1 Primer
                                3'-GTAGACAGATAGGTGACCGGGCCCTAGG-5'
421  tctcagcgcacaaacgacacccccatctgtctatccactggcccggtatcc  470
   aggagtcgggttttctgtgtggggtagacagataggtgacccgggcccctagg
   S S]

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Figure 3. Mammalian cell expression vectors used to produce chimeric and reshaped human antibodies with human kappa light chains and human gamma-1 heavy chains.

A. Light chain expression vector.



B. Heavy chain expression vector.

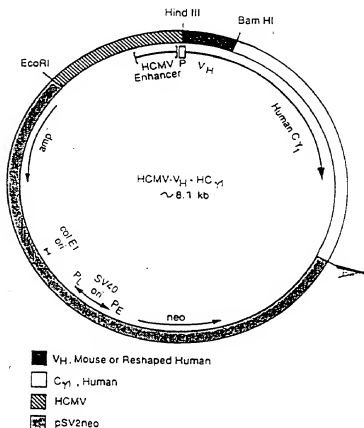


Figure 4. ELISA testing the ability of chimeric 21.6 antibody to bind to L cells expressing human $\alpha 4 \beta 1$ integrin on their surface.

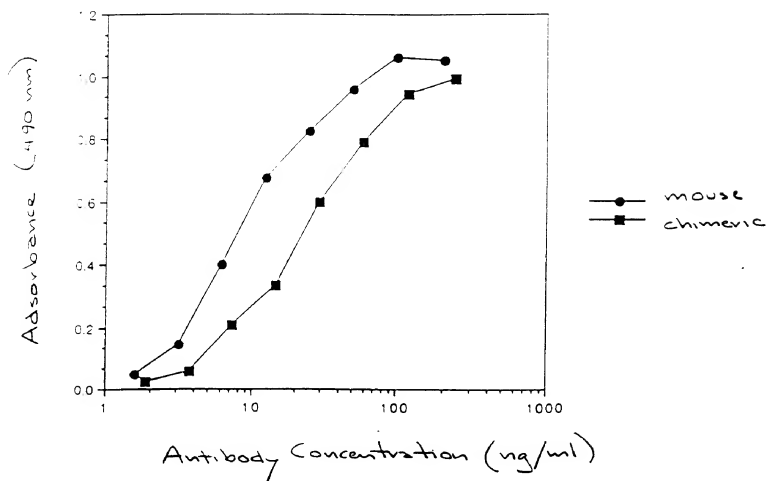


Figure 5. A view of the molecular model of the variable regions of mouse 21.6 antibody.

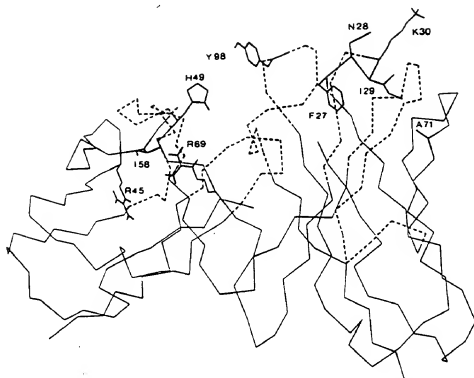


Figure 6. Comparisons of the amino acid sequences of mouse and reshaped human 21.6 light chain variable regions.

	FR1	CDR1	FR2	CDR2
	1	2	3	4
	12345678901234567890123	45678901234	567890123456789	0123456
	*	*****	*	***
21.6	DIQMTQSPSSLSASLGKVTITC	KTSQDINKYMA	WYQHKPGKRPRLLIH	YTSALQP
REI	DIQMTQSPSSLSASVGDRTITC	QASQDIKLYN	WYQQTTPGKAPKLLIY	EASNLQA
La	DIQMTQSPSSLSASVGDRTITC	KTSQDINKYMA	WYQQTTPGKAPRLLIH	YTSALQP
Lb	-----	-----	-----R-----	-----

	FR3	CDR3	FR4
	6	7	8
	7890123456789012345678	901234567	8901234567
	*	*	*****
21.6	GIPSRFSGSGSGRDYTFNISNLEPEDIATYYC	LQYDNL-WT	FGGGTKLEIK
REI	GVPSRFSGSGSGTDYTFITISLQPEDIAITYYC	QQYQSLPYT	FGQGTKLQIT
La	GIPSRFSGSGSGRDYTFITISLQPEDIAITYYC	LQYDNL-WT	FGQGT <u>KVEIK</u>
Lb	-I-----R-----	-----	-----VE-K

Figure 7. Comparisons of the amino acid sequences of the mouse and reshaped human 21.6 heavy chain variable regions.

	FR1	CDR1	FR2	CDR2
	1 2 3	4	5 6	
	123456789012345678901234567890	12345 67890123456789	012A3456789012345	
	*****	***	*****	
21.6	EVQLQQSGAELVKPGASVKLSCTASGFN	DTYIH	CVKQRPEQGLEWIG	RIDPANGYTKYDPKFG
2*CL	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYAMH	WVRQAPGQRLEWMG	WINAGNGNTKYSQKFG
Ha	QVQLVQSGAEVKKPGASVKVSCKASGFN	DTYIH	WVRQAPGQRLEWMG	RIDPANGYTKYDPKFG
Hb	-----FNIK	----	-----G----	-----
Hc	-----FNIK	----	-----	-----

	FR3	CDR3	FR4
	7 8 9	10	11
	67890123456789012ABC345678901234	567890ABCDEF12	34567890123
	*	*	
21.6	KATITADTSSNTAYLQLSSLTSED	EGYYGNYGVYAMDY	WGQGTSTVTSS
2*CL	RVTITRDTSSASTAYMELSSLRSED	GGYYGSGS----	NY WGQGTSTVTSS
Ha	RVTITADTSSASTAYMELSSLRSED	EGYYGNYGVYAMDY	WGQGTSTVTSS
Hb	-----A-----	-----	-----
Hc	-----A-----	-----F-----	-----

Figure 8. Diagram of the PCR-based construction of version "a" of reshaped human 21.6 light chain variable region.

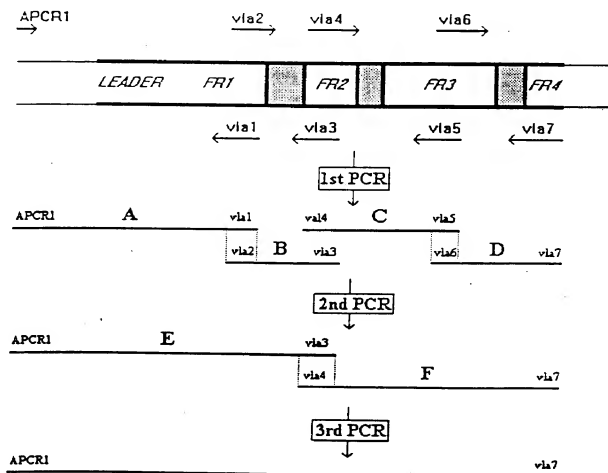


Figure 9. Diagram of the PCR-based construction of version "a" of reshaped human 21.6 heavy chain variable region.

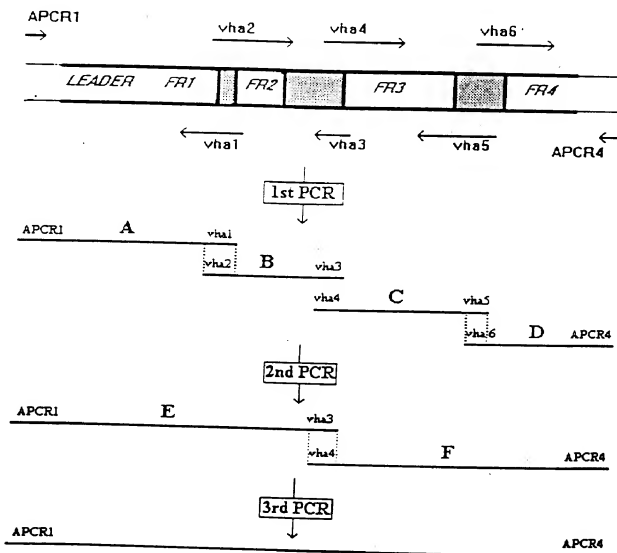


Figure 10. DNA and amino acid sequences of the first version ("a") of reshaped human 21.6 light chain variable region.

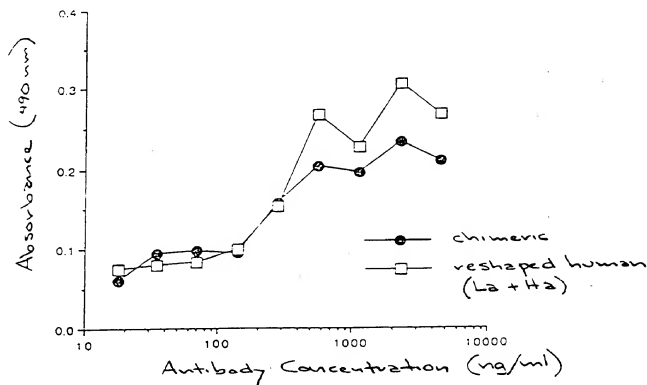
HindIII Kozak sequence
 1 aagcttgcgcaccatgagaccgtctattcagttcctgggctcttggtgtctggctt 60
 ttcgaacggcggtggtactctggcagataagtcaaggaccccgagacaacaagaccgaa
 [M R P S I Q F L G L L L F W L -
 Leader
 61 catggtgctcagtggtgacatccagatgacacagttctccatcctcactgtctgcatctGTA 120
 gtaccacaggtcacactgtaggtctactgtgtcagaggtaggagtgacagacgtagaCAT
 H G A Q C][D I Q M T Q S P S S L S A S V -
 FR1
 121 ggaGATAGAgtcaccatcacttgcaagacaagccaagacattaacaagtatatggcttgg 180
 cctCTATCTcagtggttagtgaacgtttctgttcggttctgttaattgttcataataccgaacc
 G D R V T I T C][K T S Q D I N K Y M A][W -
 CDR1
 181 taccaaCAGACAcctggaaaaGCTcctaggctgctcatatactacatctgcattacag 240
 atggttGTCGTGggaccttttCGAaggatccgacgagtatgtaattgtgtagacgtaaatgtc
 Y Q Q T P G K A P R L L I H][Y T S A L Q -
 FR2 CDR2
 241 ccaggcatcccatcaagggttcagtggaagtgggtctgggagagattatACTttcACCatc 300
 ggtccgtagggttagttccaagtcaccttcacccagaccctctctaataTGAaagTGGtag
 P][G I P S R F S G S G S G R D Y T F T I -
 FR3
 301 agcAGCctgCAGcctgaagatattgcaacttattattgtctacagtatgataatctggtg 360
 tcgTCGgacGTCggacttctataacgttgaataataacagatgtcatactattagacacc
 S S L Q P E D I A T Y Y C][L Q Y D N L W -
 CDR3
 361 acgttcggtCAAggcaccaagGTGaaatcaaacgtgagtggtacc 406
 tgcaagcccaGTTccgtgggttcCACcttttagtttgactcacctagg
 T][F G Q G T K V E I K]
 FR4

Figure 11. DNA and amino acid sequences of the first version ("a") of reshaped human 21.6 heavy chain variable region.

HindIII Kozak Sequence
 1 AAGCTTGCGGCCACCATGGACTGGACCTGGCGCGTGTTTTGGCTGCTCGCCGTGGCTCCT 60
 TTCGAACGGCGGTGGTACCTGACCTGGACCGCGCACAAAACGGACGAGCGGCACCGAGGA
 [M D W T W R V F C L L A V A P -
 Leader
 61 GGGGCCCACAGCCAGGTGCAACTAGTGCAGTCCGGCGCCGAAGTGAAGAAACCCGGTGCT 120
 CCCCGGGTGTCGGTCCACGTTGATCAGTCAGGCGCGGCTTCACTTCTTTGGGCCACGA
 G A H S][Q V Q L V Q S G A E V K K P G A -
 FR1
 121 TCCGTGAAGTCAGCTGTAAAGCTAGCGGTttcaacattaaagacacctataacacTGG 180
 AGGCACTTTCAGTCGACATTTTCGATCGCCAaagttgtatattctgtggatatatgtgACC
 S V K V S C K A S G F N I K][D T Y I H][W -
 CDR1
 181 GTTAGACAGGCCCTtGGCCAAaGGCTgGAGTGGATgGGAaggattgatcctgcgaatggt 240
 CAATCTGTCCGGGgACCGGTTtCCGAeCTCACCTAeCCTtcctaactaggacgcttacca
 V R Q A P G Q R L E W M G][R I D P A N G -
 FR2
 241 tatactaaatatgacccgaagttccaggggccgggtcACCAtcACCgcaGACACCTCTgcc 300
 atatgatttatactgggcttcaaggtcccgcccgTGGtagTGGcgtCTGTGGAGAcgg
 Y T K Y D P K F Q G][R V T I T A D T S A -
 CDR2
 301 agcACCGCCTACATGGAAGTGTCCAGCCTGCGCTCCGAGGACACTGCAGTCTACTACTGC 360
 tcgTGGCGGATGTACCTTGACAGGTCCGACGCGAGGCTCCTGTGACGTGACATGATGACG
 S T A Y M E L S S L R S E D T A V Y Y C -
 FR3
 361 GCCagagagggatattatggttaactacggggtctatgctatgGACTAcTGGGGTCAaGGA 420
 CGGtctctccctataataccattgatgccccagatacgtatgCTGATgACCCCAgTtCCT
 A R][E G Y Y G N Y G V Y A M D Y][W G Q G -
 CDR3
Splice Donor Site BamHI
 421 ACCCTTGTCACCGTCTctcTCAGGTGAGTGGATCC 454
 TGGGAACAGTGGCAGgagAGTCCACTCACCTAGG
 T L V T V S S]
 FR4

Figure 12. ELISA testing the ability of chimeric and reshaped human 21.6 antibodies to bind to L cells expressing human $\alpha 4 \beta 1$ integrin on their surface.

Panel A. Analysis of reshaped human 21.6 antibody (La + Ha).



Panel B. Analysis of reshaped human 21.6 antibody (La + Hc).

